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of the United States Geological Survey



Chapter A3

**METHODS FOR ANALYSIS OF  
ORGANIC SUBSTANCES IN WATER**

By Donald F. Goerlitz and Eugene Brown



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## PREFACE

The series of manuals on techniques describes procedures for planning and executing specialized work in water-resources investigations. The material is grouped under major subject headings called books and further subdivided into sections and chapters. The unit of publication, the chapter, is limited to a narrow field of subject matter. This format permits flexibility in revision and publication as the need arises. Section A of Book 5 presents techniques used in water analysis.

Provisional drafts of chapters are distributed to field offices of the U.S. Geological Survey for their use. These drafts are subject to revision because of experience in use or because of advancement in knowledge, techniques, or equipment. After the technique described in a chapter is sufficiently developed, the chapter is published and is for sale by the U.S. Geological Survey, 604 South Pickett Street, Alexandria, VA 22304.

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# METHODS FOR ANALYSIS OF ORGANIC SUBSTANCES IN WATER

By Donald F. Goerlitz and Eugene Brown

## Abstract

This manual contains methods used by the U.S. Geological Survey for the determination of organic substances in water. Procedures are included for the following categories of organic substances: Organic carbon, chlorophylls, color, detergents, nitrogen, oils and waxes, oxygen demand (chemical), phenolic materials, herbicides, and insecticides. Procedures are also given for the determination of chlorinated hydrocarbon insecticides, as well as chlorinated phenoxy acid herbicides, in sediment and bottom materials.

## Introduction

The yellow or brown color commonly associated with natural water is often the result of the decomposition of naturally occurring organic matter. The most abundant sources of this material include decaying vegetation, algae, and microscopic organisms. These substances are produced mostly on land and during runoff are flushed into the water, where complex biological processes continue. The role of natural organic substances in water processes is not very well understood. These compounds are known to aid in transporting and solubilizing many trace elements and are important in weathering. Further, natural organic substances interact with both organic and inorganic pollutants.

In addition to organic matter from natural sources, increasing amounts are entering water as a direct and (or) indirect result of man's activities. Leading sources of this material are industrial and domestic waste, agriculture, urban runoff, mining, and watercraft. Foaming detergents have been observed in water supplies. Fish kills have been caused by toxic chemicals and have been linked to nutrient-induced algae blooms, which deplete the dissolved oxygen.

Because of the myriad of sources from both pollution and natural processes, organic matter is present in almost all surface and ground waters and directly influences the water quality.

In reviewing water-quality reports, one may observe that although color, oxygen consumed (chemical oxygen demand), and other criteria for the analysis of organic substances are described in the introductory remarks, seldom are such data included in the tables of the report. These data are noticeably absent from past reports because such crude measurements did not appear to serve any meaningful purpose in explaining most water processes. The value of even the most primitive measurements of organic substances in water is apparent, considering the general awareness of man's effect on the environment and the implementation of programs to stem the tide of water pollution, lake eutrophication, and similar problems.

The purpose of this manual is to provide directions for collection and analysis of water samples containing organic substances as required by the Geological Survey in making water-quality investigations. This manual is an updating and compositing of the organic analytical methods contained in Geological Survey Water-Supply Paper 1454, "Methods for Collection and Analysis of Water Samples" (Rainwater and Thatcher, 1960). The intense activity in the field of organic analysis is providing new and improved methods, and as appropriate methods become available they will be added to this manual. All methods as written may be subjected to revision from time to time. Methods labeled as tentative are assumed to be adequate but need further testing before final acceptance.

## Part I. Sampling

Refer to Part I, Chapter A1 of Book 5, for information on site selection, frequency, field safety, and first aid. Before a proper sampling program can be initiated, the nature of the organic compounds sought must be considered. As a general rule, organic compounds having less than six carbon atoms are water soluble, whereas compounds with more than six carbons are not. This generalization must be applied with care, however, because the presence of hydroxyl or other polar sites on a molecule increases its solubility. Even very soluble compounds, however, may prefer to reside on organic-layered sediment. Further, some insoluble compounds may be suspended on the water surface. In summary, organic matter in a body of water may be distributed on the surface, in suspension, adsorbed on suspended sediment, in bed materials, and (or) in solution.

Because of this wide and generally unpredictable distribution of organic material in a body of water, the collection of a truly representative sample requires a great deal of care and often requires the use of specialized sampling equipment. In most instances, samples for organic analysis are best collected with the same equipment and technique used for the collection of samples for suspended-sediment measurements. The technique and equipment used in such measurements have been extensively studied, and they are discussed at length in one of a series of reports by the Inter-Agency Committee on Water Resources (1963) and, more recently, in a report by Guy and Norman (1970). When more elaborate equipment is not available, a simple weighted bottle may often be used for sampling wells, lakes, and slow-moving streams with little suspended sediment. Other available samplers have rubber gaskets and valving arrangements that may either contaminate the sample or remove organics from the water by adsorption. Such devices as the DH-48, D-49, or the P-46 samplers, which are specially designed to collect suspended-sediment samples from fast-moving streams at water velocity, must be modified for organic sample collection. Most importantly, all the neoprene gaskets must be replaced by inert plastic material, such as teflon, and oil must be eliminated from valves.

Organic substances on the water surface or incorporated in the bed material present very difficult sampling problems. Surface films may appear chronically or intermittently and most often come from accidental spills or seepage. Samples taken from the surface of a body of water, especially moving water, are almost impossible to evaluate. Quite often the samples must be related to the total volume of water, surface area, film area, and film thickness. At present, no universally acceptable surface samplers are available, and investigators are required to fabricate their own. Floating hoops or rectangles covered with clean metal foil which delineate an area and allow skimming a surface sample from calm water have been used with limited success. Other methods for collecting films and slicks from surface waters, such as "dustpan skimmers," absorbent cloths, and floor mops, have been used, but none have been satisfactory for general application. The method most often used for describing a problem area when surface films are involved is identification of the material, locating the source, and then estimating the amount entering the water.

A rational approach to site selection for sampling stream bottoms is also difficult. Fortunately, bed-material samplers are available. Ideally, bottom material, including that which is moving by saltation, rolling, or otherwise drifting along the streambed, should be included. It is also desirable to collect the water from the 3 or 4 inches of flow immediately above the stream bottom. This is commonly called the "unsampled zone," because it is not sampled by suspended-sediment samplers or most other water-sampling devices because of mechanical limitations.

Bed-material and suspended-sediment samplers may be obtained through the Federal Inter-Agency Project, U.S. Army Engineer District, St. Paul, Minn. One of these is the piston-type bed-material hand sampler BMH-53 for shallow water, and the other is the BM-54 or BM-60 bed-material sampler for deeper water. Both of these are constructed to prevent sample washout when raised to the surface. Bed-material samplers to sample at the interface are now under development and should be available soon.

Most investigations are not concerned with surface or bottom material, but rather with that within the body of the water. Most of samples are

collected from just beneath the surface of the stream to as near the bottom as the sampling apparatus will allow. Accurate samples are taken either by depth integration or at a number of points in a transverse cross section. For small streams, a depth-integrated sample or a point sample taken at a single transverse position located at the centroid of flow is usually adequate. Larger streams require selection of several verticals at centroids of equal flow after careful and often extensive measurement. Depth-integrated samples may also be taken by the ETR (equal transit rate) method, at equally spaced points across the stream cross section. In collecting these samples, the sampler is lowered and raised at each sampling point in the same length of time (Guy and Norman, 1970). Lakes are sampled on three-dimensional grids consistent with the shape and depth of the water body. Wells may be sampled from the pump, provided the oils from the mechanism do not contaminate the sample, otherwise a bottle sampler should be used. Compositing samples in the field is not recommended. Individual samples for compositing should be taken to the laboratory, where careful control can be maintained. Organic sediments and soils tend to cling to sample containers, and special precaution must be taken to avoid improper handling.

Glass bottles are the most acceptable containers for collecting, transporting, and storing samples for organic analysis. Glass appears to be inert relative to organic materials and can withstand a rigorous cleaning procedure. Because organic materials are so plentiful in the environment, it is extremely difficult to collect samples free from extraneous contamination. Apparatus for sampling or processing samples must be scrupulously clean. Sample bottles, especially, must be free of contaminating materials. Boston round glass bottles of 1-liter capacity with sloping shoulders and narrow mouths are satisfactory for most applications. The closure should be metal, preferably inert, and lined with teflon. This plastic is the only organic material that should be allowed to contact the sample.

All sample bottles, whether new or used, must be cleaned before collecting organic samples, and the following procedure is recommended: After washing in hot detergent solution and rinsing in warm tapwater, the bottles are rinsed in dilute

hydrochloric acid. Following a rinse in distilled water, the glass containers are put into an oven and heated at 300°C (Celsius) overnight. The teflon cap liners and the metal closures are washed in detergent. After rinsing with distilled water, the caps are set aside to air-dry. The liners are rinsed in dilute hydrochloric acid and then soaked in redistilled acetone for several hours and heated at 200°C overnight. When the heat treatments are completed, the bottles are removed from the oven and capped with the closure and teflon liner. The clean bottles are usually stored or shipped in a "duo-pak" container—a form-fitting expanded polystyrene case in a corrugated cardboard carton.

In actuality, the analysis begins with the collection of the sample. The sample must represent the body of water from which it was collected at the time it was taken. The importance of the sample to the final result cannot be over-emphasized. If possible, an analyst or a person directly concerned with the particular study should collect the samples. Inexperienced personnel should never be allowed to collect samples unless they are very closely supervised. If possible, the principal investigator should give personal instruction on sample collection. In all instances, detailed printed instructions should accompany each set of sample bottles. The sample containers should always be sent from the laboratory directly to the person taking the samples just before sampling. Extra or spare sample bottles should be actively inventoried and remain under the control of the laboratory.

### Sample preservation

Most water samples for organic analysis must be protected from degradation. Provisions for refrigerating or otherwise preserving the sample should be available. Icing is the most acceptable method of preserving a sample, but it is not always possible. Timing of collection should be arranged so that the sample can reach the laboratory in a minimum of time. There is no single preservative that may be added to a sample for all forms of organic analysis, but each sample must be treated according to the analytical procedure to be performed. For the determinations in this manual, the following general methods

of sample preservation should be used:

*Oxygen demand, chemical:* Add concentrated  $\text{H}_2\text{SO}_4$  (sp gr 1.84) at a rate of 2 milliliters (ml) per liter of sample.

*Carbon, inorganic:* Refrigerate at  $4^\circ\text{C}$ .

*Carbon, organic:* Add concentrated  $\text{H}_2\text{SO}_4$  at a rate of 2 ml per liter of sample and refrigerate at  $4^\circ\text{C}$ .

*Chlorophylls:* Refrigerate at  $4^\circ\text{C}$ .

*Color:* Refrigerate at  $4^\circ\text{C}$ .

*Oils and waxes:* Add concentrated  $\text{H}_2\text{SO}_4$  until the pH of the sample is below 3.0. Generally, 5 ml per liter will be sufficient.

*Surfactants:* Refrigerate at  $4^\circ\text{C}$ .

*Nitrogen:* Add 40 milligrams (mg) of  $\text{HgCl}_2$  per liter of sample and refrigerate at  $4^\circ\text{C}$ .

*Phenolic material:* Acidify sample to pH 4.0 with  $\text{H}_3\text{PO}_4$ , add 1.0 gram (g)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  per liter of sample, and refrigerate at  $4^\circ\text{C}$ .

*Herbicides:* Acidify with concentrated  $\text{H}_2\text{SO}_4$  at a rate of 2 ml per liter of sample and refrigerate at  $4^\circ\text{C}$ .

*Insecticides:* None required for chlorinated compounds.

## References

- Guy, H. P., and Norman, V. W., 1970, Field methods for the measurement of fluvial sediments: U.S. Geol. Survey Techniques Water-Resources Inv., book 3, chap. C2, 59 p.
- Rainwater, F. H., and Thatcher, L. L., 1960, Methods of collection and analysis of water samples: U.S. Geol. Survey Water-Supply Paper 1454, 301 p.
- [U.S.] Inter-Agency Committee on Water Resources, 1963, A study of methods used in measurement and analysis of sediment loads in streams, in Determination of fluvial sediment discharge: Washington, U.S. Govt. Printing Office, Rept. 14, 151 p.

## Part II. Analysis of samples

### Carbon, all forms

The procedure given affords an accurate determination of carbon in water in solution as well as in suspended matter. Both organic and inorganic carbon can be selectively measured. The organic carbon determination gives a much truer measure of the organic matter present in aqueous solution and (or) suspension than does

the chemical oxygen demand determination. This technique is not hampered by the presence of reducing substances or affected by the type of organic material being oxidized. It cannot, however, be used to replace the biological oxygen demand determination.

#### 1. Summary of method

Using a microsyringe, a fraction of a milliliter of sample is injected into a combustion tube under a sweep of oxygen gas. All the carbon in the sample is converted to carbon dioxide and measured by use of a nondispersive infrared analyzer (Van Hall and others, 1963).

#### 2. Application

Water containing carbon from about 1.0 mg/l (milligram per liter) to 1,000 mg/l may be analyzed directly. For a more sensitive procedure, the analyst is referred to the work of Menzel and Vaccaro (1964). Higher carbon concentrations must be diluted. Water containing organic particulate matter may be analyzed directly provided the largest particle diameter is  $\frac{1}{8}$ – $\frac{1}{4}$  the syringe needle diameter. Otherwise, the particles must be reduced in size by homogenizing or blending, and the resulting suspension must be fairly uniform and stable.

Depending on the sample treatment, results should be reported as follows:

2.1 Total carbon: Sample analyzed without filtration or acidification.

2.2 Total organic carbon: Sample analyzed without filtration, but acidified and purged with inert gas to remove inorganic carbon.

2.3 Dissolved carbon: Sample analyzed after filtration, but without acidification.

2.4 Dissolved organic carbon: Sample analyzed after filtration and acidification and purging to remove inorganic carbon.

In addition to these reporting forms, concentrations of inorganic carbon (dissolved and suspended) and suspended carbon (organic and inorganic) may be computed by differences from the above measurements.

#### 3. Interferences

Strongly acidic solutions and some brines interfere with this technique by producing infrared-absorbing fogs. Very volatile carbon

compounds may be lost during carbonate elimination. Injections in excess of 0.08 ml result in incomplete combustion owing to the large volume increase associated with the water vaporization.

#### 4. Apparatus

4.1 *Carbonaceous analyzer*, Beckman Model 115-A, Model 915, or equivalent.

4.2 *Microliter syringe*, 100- $\mu$ l (microliter) capacity with large-bore needle.

4.3 *Microfiltration apparatus*: Use only silver metal filters having 0.45  $\mu$ m (micrometer) maximum pore size, obtainable from Selas Flotronics. Filters should be heated at 300°C overnight to remove organic matter.

#### 5. Reagents

All reagents must be checked for organic contamination.

5.1 *Carbonate-bicarbonate standard solution*, 1.00 ml=1.00 mg carbon: Dissolve 3.500 g sodium bicarbonate and 4.418 g sodium carbonate, both dried at 105°C for 1 hr (hour), in carbon-free distilled water and dilute to 1,000 ml.

5.2 *Hydrochloric acid*, concentrated (sp gr 1.19).

5.3 *Nitrogen gas*, free of carbon dioxide and organic impurities.

5.4 *Oxygen gas*, free of carbon dioxide and organic impurities.

5.5 *Potassium hydrogen phthalate standard solution*, 1.00 ml=1.00 mg carbon: Dissolve 2.128 g potassium hydrogen phthalate, primary standard grade, which has been dried at 105°C for 1 hr, in carbon-free distilled water and dilute to 1,000 ml.

#### 6. Procedure

##### 6.A Instrument standardization

6.A.1 *Single-channel analyzer*, Beckman Model 115-A, or equivalent.

6.A.1.1 Prepare a series of standards containing 5, 10, 20, 40, 60, and 80 mg/l of carbon by dilution of the potassium hydrogen phthalate standard stock solution.

6.A.1.2 Successively introduce 20- $\mu$ l samples of the 20-mg/l standard into the carbonaceous analyzer until the response is reproducible. Allow the recorder to return to the original

baseline between injections. Run duplicate determinations, at the least, for each standard and plot the milligrams per liter of carbon versus peak height measured from baseline on the recorder.

6.A.2 *Dual-channel analyzer*, Beckman Model 915, or equivalent.

6.A.2.1 Prepare a standard curve for the organic carbon channel of the analyzer as in step 6.A.1 above.

6.A.2.2 Prepare a standard curve for the inorganic channel of the analyzer, using the same technique, by injection of standards prepared by dilution of the carbonate-bicarbonate stock standard.

##### 6.B Analysis of samples

###### 6.B.1 Single-channel analyzer

6.B.1.1 Depending on how the results are to be reported, the sample may be filtered and (or) acidified. If the sample is to be filtered through the metal membrane filter, the filtration must be done before acidification. Only enough filtrate for the determination need be collected.

6.B.1.2 Adjust the pH of the sample to 2 or below, using concentrated hydrochloric acid, and slowly bubble nitrogen gas through the solution for 3-5 min (minutes) to purge carbon dioxide.

6.B.1.3 Mix the sample well. Inject 20-80  $\mu$ l of the sample into the combustion tube, using the same technique as for the standards. Record the peak heights from at least two determinations for each sample.

6.B.1.4 Prepare a blank from carbon-free distilled water and all the reagents used, and analyze the blank as the sample. If more than 1 percent of salts is present in the sample, the blank should be prepared similarly.

###### 6.B.2 Dual-channel analyzer

6.B.2.1 Depending on whether results are desired for total or dissolved carbon, the sample may be injected untreated, or filtered through the 0.45- $\mu$ m silver filter, but *not* acidified.

6.B.2.2 Make duplicate 20- $\mu$ l injections of each sample into each channel of the analyzer, using the same technique as in the preparation of the standard curves.

#### 7. Calculations

##### 7.A Single-channel analyzer

7.A.1 Concentrations of carbon, in milligrams per liter, are obtained directly from ap-

propriate standard curves, depending on the sample treatment used.

#### 7.B *Dual-channel analyzer*

7.B.1 Concentrations of inorganic and inorganic-plus-organic carbon, in milligrams per liter, in each sample are obtained from the appropriate standard curves.

7.B.2 By difference, compute the concentration of organic carbon, in milligrams per liter. When this difference is small and the determined values are large, results should be verified. This may be done by injecting an acidified, nitrogen-purged sample into the high-temperature furnace to obtain a direct measure of organic carbon.

#### 8. Report

Carbon concentrations are reported as follows: Less than 10 mg/l, one significant figure; 10 mg/l and above, two significant figures.

#### 9. Precision

No precision data are available, but results are believed reproducible within  $\pm 1$  mg/l at the 100-mg/l level.

#### References

- Beckman Instruments, Inc., 1966, Laboratory carbonaceous analyzer: Beckman Instructions 137879, Fullerton, Calif., 27 p.
- 1968, Total organic carbon analyzer: Beckman Instructions 81706-B, Fullerton, Calif., 34 p.
- Menzel, D. W., and Vaccaro, R. F., 1964, The measurement of dissolved organic and particulate carbon in seawater: *Limnology and Oceanography*, v. 9, p. 138-142.
- Van Hall, C. E., Safranko, John, and Stenger, V. A., 1963, Rapid combustion method for the determination of organic substances in aqueous solutions: *Anal. Chemistry*, v. 35, p. 315-319.

## Chlorophylls

The concentrations of photosynthetic pigments in natural waters vary with time and with changing aquatic conditions. Chlorophyll *a*, *b*, and *c* concentrations are used to estimate the biomass and the photosynthetic capacity of phytoplankton. Ratios between the different forms of chlorophyll are thought to indicate the taxonomic composition or the physiological state of the algal community.

## Extractive spectrophotometric method

#### 1. Summary of method

Individual chlorophylls are determined simultaneously without elaborate separation. The sample is filtered, and the cells retained on the filter are mechanically disrupted to facilitate extraction of pigments into a solvent composed of 90 percent acetone and 10 percent water (volume per volume). The concentrations of chlorophylls are calculated from measurements of absorbance of the extract at 4 wavelengths, corrected for the 90-percent acetone blank.

#### 2. Application

The method is suitable for all natural waters. The volume of water filtered should contain less than 10  $\mu\text{g}$  (micrograms) chlorophyll *a*. (See step 6.1.)

#### 3. Interferences

Large amounts of inorganic sediment in the sample may clog the filter. Erroneously high values may result from the presence of fragments of tree leaves or other terrestrial plant material.

The presence of phaeopigments, the decomposition products of chlorophyll, results in overestimates of chlorophylls (Yentsch, 1965, 1969; Lorenzen, 1965, 1967).

#### 4. Apparatus

4.1 *Filtration equipment*: Filter holder assembly, Millipore XX63 001 20, or equivalent, and a source of vacuum.

4.2 *Glass pestle-type tissue homogenizer* (grinder), 15-ml capacity, Corning 7725, or equivalent. Motor drive with low-torque clutch to operate at about 500 rpm (revolutions per minute).

4.3 *Membrane filter*, Millipore HAWP 047 00 type, HA 0.45- $\mu\text{m}$  mean pore size, white, plain, 47-mm (millimeter) diameter, or equivalent.

4.4 *Swing-out centrifuge*, 4,000-5,000 gravity, with stoppered 15-ml graduated centrifuge tubes. Saveguard centrifuge, Model CT-1140, or equivalent.

4.5 *Spectrophotometer* (Beckman Model DU, or equivalent), with a bandwidth of 3 nm (nanometers) or less, allowing absorbance to be read to  $\pm 0.001$  units. Use cells with a light-path of from

1 to 10 cm (centimeters). If only chlorophyll *a* is determined, instruments with interference filters with not more than 5–10 nm (Beckman Model B, or equivalent) half-bandwidth may be used.

4.6 *Vacuum-pressure pump*, Millipore XX60 000 00, or equivalent.

### 5. Reagents

5.1 *Acetone* solution: To 900 ml acetone, add 100 ml distilled water.

5.2 *Magnesium carbonate*, powdered.

### 6. Procedure

6.1 Collect a sample of water which contains less than 10  $\mu\text{g}$ , and preferably about 1  $\mu\text{g}$ , of chlorophyll *a*. A sample of 0.5–1.0 liter may be adequate for fresh or estuarine waters; 4–5 liters of ocean water may be required. Samples must be refrigerated at 4°C until time of analysis.

6.2 Cover the surface of a 0.45- $\mu\text{m}$  membrane filter with finely powdered  $\text{MgCO}_3$ , using about 10  $\text{mg}/\text{cm}^2$  of filter area (about 170 mg for the 47-mm-diameter filter).

6.3 Filter the sample at no more than two-thirds atm (atmosphere).

6.4 Fold the filter with the plankton on the inside and proceed immediately with the extraction and measurement steps. If the sample must be stored, dry the filter in a silica gel desiccator in the dark at 1°C or less. Dry ice is recommended for storing samples in the field.

6.5 Place the filter in a glass homogenizer. Add 2 to 3 ml acetone solution. Grind 1 min (minute) at about 500 rpm.

6.6 Transfer to a graduated centrifuge tube and wash the pestle and homogenizer two or three times with acetone solution. Adjust the total volume to some convenient value, such as 5 or 10 ml  $\pm 0.1$  ml. Keep 10 min in the *dark* at room temperature.

6.7 Centrifuge for 10 min at 4,000 to 5,000 gravity.

6.8 Carefully pour or pipet the supernatant into the spectrophotometer cell. Do not disturb the precipitate. If extract is turbid, try to clear by adding a little 100-percent acetone or distilled water, or by recentrifuging.

6.9 Read the absorbance at 750, 663, 645, and 630 nm against an acetone-solution blank. (Dilute with acetone solution if the absorbance is

greater than 0.8.) If the 750-nm reading is greater than 0.005/cm light-path, reduce the turbidity as mentioned in step 6.8.

### 7. Calculations

Subtract the absorbance at 750 nm from the absorbance at 663, 645, and 630 nm. Divide the difference by the light-path of the cells, in centimeters. The concentrations of chlorophylls in the extract, as  $\mu\text{g}/\text{ml}$  (micrograms per milliliter), are given by the following equations:

$$\begin{aligned} \text{chlorophyll } a, \text{ in } \mu\text{g}/\text{ml} &= 11.64e_{663} - 2.16e_{645} + 0.10e_{630} \\ \text{chlorophyll } b, \text{ in } \mu\text{g}/\text{ml} &= -3.94e_{663} + 20.97e_{645} - 3.66e_{630} \\ \text{chlorophyll } c, \text{ in } \mu\text{g}/\text{ml} &= -5.53e_{663} - 14.81e_{645} + 54.22e_{630} \end{aligned}$$

where:

$$e_{663} = \frac{\text{absorbance at 663 nm} - \text{absorbance at 750 nm}}{\text{light-path, in cm}}$$

$$e_{645} = \frac{\text{absorbance at 645 nm} - \text{absorbance at 750 nm}}{\text{light-path, in cm}}$$

$$e_{630} = \frac{\text{absorbance at 630 nm} - \text{absorbance at 750 nm}}{\text{light-path, in cm}}$$

$$\begin{aligned} \text{chlorophyll } a, \text{ in } \mu\text{g}/\text{l} &= \text{chlorophyll } a \text{ in } \mu\text{g}/\text{ml} \\ &\times \frac{\text{extract volume, in ml}}{\text{sample volume, in liters}} \end{aligned}$$

### 8. Report

Report chlorophyll concentrations as follows: Less than 1.0  $\mu\text{g}/\text{l}$  (micrograms per liter), one decimal; 1.0  $\mu\text{g}/\text{l}$  and above, two significant figures.

### 9. Precision

The precision of chlorophyll determinations is influenced by the volume of water filtered, the range of chlorophyll values encountered, the volume of extraction solvent, and the light-path of the spectrophotometer cells.

The precision for chlorophyll *a* determination at the 5- $\mu$ g level was reported to be  $\pm 5$  percent in one study (Strickland and Parsons, 1960). The UNESCO (1966) report recommended that precision be determined by each analyst.

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## Color

The color of water as considered herein is that due only to substances in solution. Color in water may be of natural mineral, animal, or vegetable origin. It may be caused by metallic substances, humus material, peat, algae, weeds, or protozoa. Industrial wastes may also color water. Color may range from zero to several hundred units.

In domestic water, any noticeable color is undesirable. Color-imparting solutes may dull clothes or stain food and fixtures. The U.S. Public Health Service (1962) stated that the color shall not exceed 15 units in drinking and culinary water on carriers subject to Federal quarantine regulations. Color is undesirable in water for many industries, particularly food processing, laundering, ice manufacturing, bottled beverage, photographic, and textile (California State Water Quality Control Board, 1963).

### Comparison method

#### 1. Summary of method

The color of the water is compared with that of colored glass disks which have been calibrated

to correspond to the platinum-cobalt scale of Hazen (1892). The unit of color is that produced by 1 mg of platinum per liter. A small amount of cobalt is added to aid in color matching. The Hazen scale is satisfactory for most waters, but the hues and shades of some waters may not easily be compared with standards. If the hue of the water does not compare with that of the standard, there is very little that can be done, other than to visually compare the optical densities of the sample and standard. Highly colored waters should not be diluted more than necessary because the color of the diluted sample often is not proportional to the dilution. The colored glass disks should be recalibrated at frequent intervals against platinum-cobalt standards, as their color may fade with time.

#### 2. Application

This method may be used to measure the color of samples whose colors reasonably match the Hazen scale and which contain no excessive amount of suspended matter.

#### 3. Interferences

Turbidity generally causes the observed color to be higher than the true color, but there is some disagreement as to the magnitude of the effect of turbidity. The removal of turbidity is a recurrent problem in the determination of color. Color is removed by adsorption on suspended material. Filtration of samples to remove turbidity frequently removes some of the color-imparting solutes, possibly by adsorption on the sediments or on the filter medium. Centrifuging is preferable to filtration and is the only recommended method for the removal of suspended matter.

#### 4. Apparatus

*Color comparator*, with standard color disks covering the range 0-500 color units.

#### 5. Reagents

None.

#### 6. Procedure

6.1 Remove turbidity by centrifuging the sample.

6.2 Fill one instrument tube with the sample of water, level the tube, insert the glass plug,

making sure that no air bubbles are trapped, and insert the tube into the comparator.

6.3 Use distilled or demineralized water in the second tube as a blank.

6.4 The color comparison is made by revolving the disk until the colors of the two tubes match.

#### 7. Calculations

The color is read directly from the matching color standard, and the proper dilution factor is applied.

#### 8. Report

Report color as follows:

<i>Color unit</i>	<i>Record units to nearest</i>
1-50.....	1
51-100.....	5
101-250.....	10
251-500.....	20

#### 9. Precision

Because of the many complicating factors involved, the measurement of color is not a precise determination. No statements on the reproducibility of the tests can be made.

#### References

- California State Water Quality Control Board, 1963, Water quality criteria: Pub. 3-A, p. 168.  
 Hazen, Allen, 1892, A new color standard for natural waters: Am. Chem. Soc. Jour., v. 12, p. 427.  
 U.S. Public Health Service, 1962, Drinking water standards: Public Health Service Pub. 956, p. 6.

## Extractable organic matter

### Oils and waxes

Oils and waxes in natural waters most likely come from vegetation and aquatic life. Oils or fats and waxes from plants and animals are, for the most part, of the ester type—that is, the combination of an alcohol with an organic acid. Petroleum oils and tars, otherwise known as mineral oils, are almost exclusively hydrocarbon in composition. Petroleum oils, on occasion, can enter the water from natural seeps but most often result from industrial pollution and accidental

spills (Dept. of Interior, Office of Secretary, 1968). Most oils are insoluble in water but may be dispersed by natural and waste chemicals such as soaps and detergents. Ester-type compounds can hydrolyze to become soluble, form soaps, and further aid dispersion of the insoluble material. Oils, greases, fats, and waxes can severely damage water quality by (1) producing a visible film on the surface, (2) imparting an odor to the water and causing a noxious taste, (3) coating the banks and bottoms of the water body by adsorbing on sediment, and (4) destroying aquatic life (Federal Water Pollution Control Administration, 1968).

#### 1. Summary of method

Oils and waxes are removed from the water by extraction with organic solvents and are determined gravimetrically. This method is similar in principle to that found in "Standard Methods," 12th edition (Am. Public Health Assoc., 1965). For characterization, and for volatile substances, the analyst is referred to the papers by Kawahara (1969) and Machler and Greenber (1968).

#### 2. Application

The extraction method is applicable to the analysis of waters containing oil, fat, grease, wax, and other solvent-soluble substances.

#### 3. Interferences

Organic solvents vary considerably in their ability to dissolve not only oily substances but other organic matter as well. The ester-type oils and waxes may be decomposed to alcohols and acids or soaps. Glycerine, the alcohol from animal or vegetable fats and oils which are known as triglycerides, defies extraction by this technique. Soaps must be acidified to release the organic part of the molecule. Storage of the sample must be avoided because many oils are utilized by micro-organisms. Volatile compounds (boiling point  $<100^{\circ}\text{C}$ ) cannot be accurately determined by this method.

#### 4. Apparatus

4.1 *Kuderna-Danish concentration apparatus*, 250-ml capacity. Use an ungraduated receiver and a 1-ball Snyder column.

4.2 *Separator funnel*, 1-liter capacity, with unlubricated glass or teflon stopcock.

### 5. Reagents

5.1 *Chloroform*, boiling range 61°–62°C, distilled in glass.

5.2 *Petroleum ether*, boiling range 30°–60°C, distilled in glass.

5.3 *Sodium sulfate*, anhydrous, ACS reagent, granular.

5.4 *Sulfuric acid*, 1:1 solution: Mix a volume of sulfuric acid (sp gr 1.84) with an equal volume of distilled water.

### 6. Procedure

Surface samples must be taken with great care. (See p. 2.) The relationship of the affected surface to the body of water should be well documented. A complete written description of the sampling technique should be made for future reference. Samples of the water beneath the surface should be collected according to the recommended practice for organic samples. Usually 1-liter samples are sufficient for subsurface sampling.

6.1 Weigh the water and sample container with the cap removed. Pour the sample into the separatory funnel. Weigh the drained sample bottle and record the weight of the sample to three significant figures. (If an oil separation is observed, the weight of water may be obtained after a preliminary petroleum ether extraction before acidification.)

6.2 Rinse the sample bottle with 15 ml of petroleum ether and pour the washings into the separatory funnel. Rinse the sample bottle again with another 15 ml of petroleum ether and pour the washings into the separatory funnel.

6.3 Acidify the sample by the addition of 5.0 ml of 1:1 H<sub>2</sub>SO<sub>4</sub> per liter of sample.

6.4 Shake the separatory funnel vigorously for 1–2 min, stopping to vent the pressure after the first few shakes. Allow the layers to separate for about 10 min. Draw off the water layer into the sample container and pour the solvent into a 125-ml erlenmeyer flask containing about 0.5 g anhydrous sodium sulfate. Difficult emulsions may be broken by shaking the funnel vigorously after most of the water has been withdrawn. Beware of excessive pressure buildup in the funnel at this step.

6.5 Pour the sample back into the separatory funnel. Rinse the sample bottle with 15 ml of

chloroform and add the washings to the separatory funnel. Pour an additional 15 ml of chloroform into the separatory funnel and shake 1–2 min. Allow the layers to separate. Collect the chloroform layer in the 125-ml erlenmeyer flask containing the petroleum ether extract.

6.6 Filter the extract through a plug of extracted glass wool into a Kuderna-Danish apparatus fitted with a tared (including a small boiling stone) receiver tube. Use liberal washings of petroleum ether to complete the transfer.

6.7 Remove the solvent by distillation on a steam bath and take nearly to dryness. As the last of the solvent is vaporizing, remove the apparatus from the heat and allow the vapor to condense and wash down the sides of the flask. After cooling, remove the receiver and volatilize the last of the solvent with a gentle stream of air or nitrogen gas.

6.8 Wipe the sides of the receiver tube with a moist tissue and allow to dry in the room for 30 min. Place the receiver in a desiccator for 30 min and then determine the weight of the sample.

### 7. Calculations

The concentration of material in the sample is calculated as follows:

$$\begin{aligned} \text{Extractable organic matter (mg/l)} \\ = \frac{A - B}{C} \times 1,000, \end{aligned}$$

where

$A$  = total weight (receiver + extract), in grams,  
 $B$  = receiver tare, in grams, and  
 $C$  = water sample volume, in liters.

The amount of material found in a surface sample may be related to an affected area provided the film is uniform. The weight of material per square meter of surface may be calculated as follows:

$$\text{Extractable organic matter (mg/m}^2\text{)} = \frac{1}{A} \times B,$$

where

$A$  = area sampled, in square meters (m<sup>2</sup>), and  
 $B$  = weight of extracted material, in milligrams.

### 8. Report

Concentrations of extractable organic matter are reported to two significant figures for values in excess of 10 mg/l, or to the nearest whole milligram.

### 9. Precision

The precision between samples depends upon the homogeneity of the dispersion and the volatility of the material. Duplicate results varying less than 5 percent from the mean have been obtained on prepared samples. Results from poorly dispersed material and surface film samples vary greatly.

### References

- American Public Health Association, 1965, Standard methods for the examination of water and wastewater [12th ed.]: New York, Am. Public Health Assoc., Inc., 769 p.
- Kawahara, F. K., 1969, Laboratory guide for the identification of petroleum products: Cincinnati, Federal Water Pollution Control Admin., 41 p.
- Maehler, C. A., and Greenber, A. E., 1968, Identification of petroleum in estuarine waters: Am. Soc. Civil Eng. Proc., Jour. Sanitary Eng. Div., v. 94, No. SA5, p. 969-978.
- [U.S.] Department of the Interior, Office of the Secretary, 1968, Oil pollution; A report on pollution of the Nation's waters by oil and other hazardous substances: Washington, U.S. Govt. Printing Office, 31 p.
- [U.S.] Federal Water Pollution Control Administration, 1968, Report of the committee on water-quality criteria: Washington, U.S. Govt. Printing Office, 234 p.

## Methylene blue active substances

### Synthetic anionic detergents

Methylene blue active substances (MBAS), synthetic detergents or surfactants, occur in natural waters almost exclusively as a result of pollution. Detergents in natural water can drastically alter the normal regimen. As surface-active agents, they disrupt the stability at the interface between water and other substances. In the presence of surfactants, normally insoluble organic compounds may be dispersed, and the function of vital membranes of aquatic organisms may be altered. Because they are water soluble, detergents can disperse toxic organic compounds normally incorporated in bottom muds, adsorbed

on sediment, or floating on the surface. In addition to acting synergistically to compound pollution problems, some detergents are toxic to certain aquatic life. According to the report of the Federal Water Pollution Control Administration (1968) levels of LAS (linear alkylate sulfonates) exceeding 0.2 mg/l may be harmful to aquatic life.

Three types of surface-active agents are in general use. They are classified by their chemical characteristics as anionic or cationic, indicating the charge of the active ions, and as nonionic hydrophilic organic compounds. Fortunately, anionic detergents react with cationic detergents to precipitate. Production of anionic detergents has greatly exceeded the other two, and, consequently, greater amounts of anionic detergents may be expected in waste water. Owing to this excess, cationic detergents are almost always effectively precipitated and seldom appear in solution in sewage treatment facilities. Synthetic nonionic detergents, alkyl or aryl polyols, have not caused much concern because their use has been limited principally to industrial applications and as a minor additive in household detergents for foam control.

Until 1965, ABS (alkyl benzene sulfonates) anionic detergents were the most common surfactants on the market. Because ABS resist biological degradation, they are not easily removed by waste treatment and were often found in water supplies, even drinking water. Since then, LAS (linear alkyl sulfonates), for the most part, have replaced ABS in detergent formulation because LAS surfactants are biodegradable and may be removed from waste water by ordinary treatment.

Both ABS and LAS are methylene blue active substances but are not identical in color production for spectrophotometric determination. Owing to this, methylene blue active substances are measured in units relative to an ABS standard.

### 1. Summary of method

Methylene blue reacts with anionic surfactants, both ABS and LAS, to form a blue-colored dye complex. The complex is extracted in chloroform, and the methylene blue active substances are determined spectrophotometrically. This method is similar in substance to "Standard Methods," 12th edition (Am. Public Health Assoc., 1965).

## 2. Application

This method is applicable to the analysis of waters containing 0.025–100 mg/l MBAS (methylene blue active substances relative to ABS standard).

## 3. Interferences

Phenols, proteins, and inorganic chlorides, cyanates, nitrates, and thiocyanates will complex methylene blue and give positive interference in the determination. With ABS concentrations from 0.0 to 1.0 mg/l, tests have shown no interference from the following individual constituents: 10 mg/l NO<sub>2</sub>, 25 mg/l NO<sub>3</sub>, 5 mg/l phenol, and 1 mg/l H<sub>2</sub>S. Organic compounds having amine groups cause low results.

## 4. Apparatus

4.1 *Separatory funnels*, 500-ml, unlubricated glass or teflon stopcock.

4.2 *Spectrophotometer*, Beckman Model B, or equivalent.

With this instrument the following conditions have been used:

Wavelength.....	635 nm.
Cells.....	10 mm.
Phototube.....	Red-sensitive.
Initial sensitivity setting.....	2.
Slit width.....	0.2 mm.
Blank.....	CHCl <sub>3</sub> .

With these operating conditions, the following absorbances have been observed:

MBAS (as ABS) (mg)	Absorbance
0.01.....	0.052
.02.....	.150
.05.....	.438
.10.....	.870

## 5. Reagents

5.1 *Chloroform*, spectrophotometric grade.

5.2 *Detergent* (ABS) standard solution I: Dissolve 1.030 g 97.1-percent-purity ABS powder (obtained from Association American Soap and Glycerine Producers, Inc., 295 Madison Ave., New York, N. Y. 10010, and dried over H<sub>2</sub>SO<sub>4</sub> for 1 week) in 500 ml distilled water in a 1,000-ml volumetric flask with gentle swirling. Allow the foam to break and dilute to volume. Refrigerate during storage.

5.3 *Detergent* standard solution II, 1.00 ml=0.010 mg ABS: Dilute 10.0 ml detergent standard solution I to 1,000 ml. Prepare fresh weekly.

5.4 *Methylene blue reagent*: Dissolve 0.35 g methylene blue in 0.01N sulfuric acid and dilute to 1,000 ml with same.

5.5 *Sulfuric acid* solution, 5N: Mix 245 ml concentrated H<sub>2</sub>SO<sub>4</sub> (sp gr 1.84) in 500 ml water and, after cooling, dilute to 1,000 ml.

## 6. Procedure

Samples should be collected according to the recommended practice for organic analysis. Usually a 1-liter sample is adequate. LAS detergents are biodegradable; therefore samples should be analyzed as soon as possible after collection. All glassware should be rinsed with dilute hydrochloric acid after washing. As a further precaution, sample bottles, separatory funnels, and beakers may be heat treated at 300°C overnight to remove organic matter.

6.1 Measure a volume of sample containing less than 0.10 mg MBAS and not to exceed 100 ml into separatory funnel. Dilute to 100 ml if necessary. Prepare a blank and detergent standards in the same manner.

6.2 Adjust the pH of the sample to near neutral if necessary. To the sample, blank, and standard solution, add 1.0 ml 5N H<sub>2</sub>SO<sub>4</sub>, mix, then add 5.0 ml methylene blue solution. Mix thoroughly.

6.3 Add 25.0 ml chloroform and shake the contents of the separatory funnel for 1 min. Allow the layers to separate.

6.4 Drain the lower chloroform layer through a layer of absorbent cotton contained in a small filtering funnel into 1.0-cm cells and measure the absorbance of the sample and standards against the blank at a wavelength of 655 nm.

## 7. Calculations

Determine the amount of detergent contained in the sample, minus the blank, from the standard curve. Calculate the amount of MBAS in the sample using the following equation:

$$\begin{aligned} & \text{MBAS (relative to ABS standard) mg/l} \\ &= \frac{\text{mg MBAS}}{\text{ml sample}} \times 1,000 \end{aligned}$$

**8. Report**

Report synthetic anionic detergents concentrations as follows: Less than 1 mg/l, two decimals; greater than 1 mg/l, two significant figures.

**9. Precision**

Deviation of  $\pm 10$  percent may be expected in the range of 1–5 mg/l in surface water.

**References**

- American Public Health Association, 1965, Standard methods for the examination of water and wastewater [12th ed.]: New York, Am. Public Health Assoc., Inc., 769 p.
- [U.S.] Federal Water Pollution Control Administration, 1968, Report of the committee on water-quality criteria: Washington, U.S. Govt. Printing Office, 234 p.

**Nitrogen, ammonia**

Ammonia nitrogen includes nitrogen in the forms of  $\text{NH}_3$  and  $\text{NH}_4^{+1}$ . As a component of the nitrogen cycle, it is often present in water, but usually in only small amounts. Ammonia is used in some water-treatment processes. More than 0.1 mg/l usually indicates organic pollution (Rudolph, 1931).

There is no evidence that ammonia nitrogen in water is physiologically significant to man or livestock. Fish, however, cannot tolerate large quantities. The toxicity to fish is directly related to the amount of free ammonia in solution; hence, the toxicity is dependent on the pH of the water. Ammonia decreases the ability of hemoglobin to combine with oxygen, and the fish suffocate. Although the tolerances of fish differ, 2.5 ml/l of ammonia nitrogen is considered harmful in the 7.4–8.5 pH range (Ellis and others, 1948).

The low concentrations of ammonia in natural waters are of little industrial significance, except that ammonium salts are destructive to concrete.

**Distillation method****1. Summary of method**

The sample is buffered to a pH of 9.5 to minimize hydrolysis of organic nitrogen compounds. Ammonia is distilled from the buffered solution,

and an aliquot of the distillate is then Nesslerized. Essentially, Nesslerization is the reaction between potassium mercuric iodide and ammonia which forms a red-brown complex of mercuric ammono-basic iodide:



Concentrations of ammonia are then determined by standard spectrophotometric measurements. Alternatively, the distillate may be titrated with standard sulfuric acid solution.

Additional information on the principle of the determination is given by Kolthoff and Sandell (1952, p. 633) and by Blaedel and Meloche (1963).

**2. Application**

This method is recommended for analysis of samples containing less than 2 mg of ammonia and ammonium ion per liter. Higher concentrations may be determined by the alternate titration procedure provided.

**3. Interferences**

Calcium, magnesium, iron, and sulfide interfere with the Nesslerization, but the interference of the metals is eliminated by the distillation, and sulfide can be precipitated in the distillation flask with a little lead carbonate.

Some organic compounds may distill over with the ammonia and form colors with Nessler reagent which cannot be satisfactorily read with the spectrophotometer. Under such conditions, the sample should be distilled into  $\text{H}_3\text{BO}_3$  and titrated with standard  $\text{H}_2\text{SO}_4$ .

**4. Apparatus**

4.1 *Kjeldahl distillation apparatus*, 1,000-ml flasks.

4.2 *Spectrophotometer*, Beckman Model B, or equivalent.

With this instrument, the following operating conditions have been used:

Wavelength.....	425 nm.
Cells.....	40 mm.
Phototube.....	Blue-sensitive.
Initial sensitivity setting....	1.
Slit width.....	0.3 mm.
Blank.....	Ammonia-free water plus reagents.

With these operating conditions, the following absorbances have been observed:

<i>Nitrogen</i> (mg)	<i>Absorbance</i>
0.02-----	0.24
.04-----	.47
.06-----	.70
.10-----	1.16

#### 5. Reagents

5.1 *Ammonium chloride* standard solution I, 1.00 ml = 1.00 mg N: Dissolve 3.819 g  $\text{NH}_4\text{Cl}$ , dried overnight over sulfuric acid, in ammonia-free water and dilute to 1,000 ml.

5.2 *Ammonium chloride* standard solution II, 1.00 ml = 0.010 mg N: Dilute 10.00 ml ammonium chloride standard solution I to 1,000 ml with ammonia-free water. Prepare fresh.

5.3 *Borate* buffer solution: Dissolve 9.54 g  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  in ammonia-free water. Adjust the pH to 9.5 with 1M NaOH (approx 15 ml) and dilute to 1 liter with ammonia-free water.

5.4 *Boric acid* solution: Dissolve 20 g  $\text{H}_3\text{BO}_3$  in ammonia-free water and dilute to 1 liter.

5.5 *Methyl red indicator* solution: Dissolve 0.1 g methyl red indicator in 100 ml ethanol.

5.6 *Nessler* reagent (CAUTION:  $\text{HgI}_2$  is a deadly poison, and the reagent must be so marked): Dissolve 100 g  $\text{HgI}_2$  and 70 g KI in a small volume of ammonia-free water. Add this mixture slowly, with stirring, to a cooled solution of 160 g NaOH in 500 ml ammonia-free water and dilute to 1 liter. Allow the reagent to stand at least overnight and filter through a fritted-glass crucible.

5.7 *Sodium carbonate* solution, 0.0357N: Dissolve 1.892 g primary standard  $\text{Na}_2\text{CO}_3$  in carbon dioxide free water and dilute to 1,000 ml.

5.8 *Sodium hydroxide* solution, 1M: Dissolve 40 g NaOH in ammonia-free water and dilute to 1 liter.

5.9 *Sulfuric acid* standard solution, 0.0357N, 1.00 ml = 0.5 mg N: Mix 1.3 ml concentrated  $\text{H}_2\text{SO}_4$  (sp gr 1.84) with demineralized water and dilute to 950 ml before standardization. Standardize by titrating 25.0 ml 0.0357N  $\text{Na}_2\text{CO}_3$  to pH 4.5.

#### 6. Procedure

If acid has been added to the sample as a preservative at the time of collection, this must

be neutralized with NaOH solution before proceeding with the analysis. All glassware should be rinsed with ammonia-free water, prepared by passing distilled water through a mixed-bed ion-exchange resin.

6.1 Free the distillation apparatus of ammonia by boiling ammonia-free water until the distillate shows no trace using Nessler reagent (CAUTION: Deadly poison).

6.2 Measure a volume of sample containing less than 1.0 mg ammonia nitrogen (500 ml maximum) into a 600-ml beaker, and adjust the volume to approximately 500 ml with ammonia-free water.

6.3 Add 25 ml borate buffer solution and adjust the pH to 9.5 with 1M NaOH, if necessary.

6.4 Immediately transfer the solution into the distillation flask and distill at a rate of 6–10 ml per minute; catch the distillate in a 500-ml volumetric flask containing 50 ml boric acid solution. The tip of the delivery tube must be below the surface of the boric acid.

6.5 Collect approximately 250 ml of distillate, dilute to 500 ml with ammonia-free water, and mix.

#### 6.6.A Nesslerization procedure

6.6.A.1 Pipet an aliquot of distillate containing less than 0.1 mg ammonia nitrogen (50.00 ml maximum) into a graduate, and adjust the volume to 50.0 ml with ammonia-free water.

6.6.A.2 Prepare a blank of ammonia-free water and sufficient standards. Add 5 ml boric acid solution to each, and adjust the volumes to 50.0 ml.

6.6.A.3 Add 1.0 ml Nessler reagent (CAUTION: Deadly poison), and mix.

6.6.A.4 Allow the solutions to stand at least 10 min, but not over 30 min.

6.6.A.5 Determine absorbance of test sample and standards against the blank.

#### 6.6.B Titration procedure

6.6.B.1 To the distillate, and an ammonia-free water blank containing the same volume of  $\text{H}_3\text{BO}_3$ , add 3 drops methyl red indicator solution, and titrate with sulfuric acid standard solution.

#### 7. Calculations

#### 7.A Nesslerization procedure

7.A.1 Determine mg N in aliquot from a plot of absorbances of standards.

## 7.A.2 Ammonia nitrogen as N, in mg/l

$$= \frac{1,000}{\text{ml sample}} \times \frac{500}{\text{ml aliquot}} \times \text{mg N in aliquot.}$$

## 7.B Titration procedure

## 7.B.1 Ammonia nitrogen as N, in mg/l

$$= \frac{V_a \times N_a \times 14,000}{V_s},$$

where

$V_a$  = ml standard  $\text{H}_2\text{SO}_4$  used in titration of sample minus ml used to titrate blank,

$N_a$  = normality of standard  $\text{H}_2\text{SO}_4$ , and

$V_s$  = ml of original sample used for distillation.

7.C Ammonia nitrogen as  $\text{NH}_4^{+1}$ , in mg/l = mg/l as  $\text{N} \times 1.288$ . Ammonia nitrogen as free  $\text{NH}_3$ , in mg/l = mg/l as  $\text{N} \times 1.216$ .

## 8. Report

Report ammonia nitrogen concentrations as follows: Less than 1 mg/l, two decimals; 1 mg/l and above, two significant figures.

## 9. Precision

No precision data are available.

## References

- Blaedel, W. J., and Meloche, V. W., 1963, *Elementary quantitative analysis—Theory and practice* [2d ed.]: New York, Harper & Row, 826 p.
- Ellis, M. M., Westfall, B. A., and Ellis, M. D., 1948, *Determination of water quality: U.S. Fish Wildlife Service Research Rept. 9*, 122 p.
- Kolthoff, I. M., and Sandell, E. B., 1952, *Textbook of quantitative inorganic analysis* [3d ed.]: New York, Macmillan Co., 759 p.
- Rudolph, Z., 1931, *Principles of the determination of the physical and chemical standards of water for drinking, industrial, and domestic purposes: Water Pollution Abs. 4* [March].

## Nitrogen, nitrate

Nitrate is usually the most prevalent form of nitrogen in water because it is the end product of the aerobic decomposition of organic nitrogen. Nitrate from natural sources is attributed to the oxidation of nitrogen of the air by bacteria and to the decomposition of organic material in the soil. Fertilizers may add nitrate directly to water resources. Nitrate concentrations range from a

few tenths to several hundred milligrams per liter, but in unpolluted water they seldom exceed 10 mg/l. Nitrate and chloride are major components of human and animal wastes, and abnormally high concentrations of both suggest pollution.

Cyanosis due to methemoglobinemia may occur in infants whose drinking or formula water contains a high concentration of nitrates. The nitrates, when ingested, are converted to nitrites in the digestive system of some infants. The nitrite ion oxidizes hemoglobin to methemoglobin and thereby causes cyanosis. It is widely recommended that water containing more than 10–20 mg/l of nitrate nitrogen should not be used in infant feeding (Comly, 1945).

Nitrates in large amounts are injurious to the dyeing of wool and silk and are undesirable in fermentation processes (California State Water Quality Control Board, 1963). At least 2 mg/l of nitrate prevents intercrystalline cracking of steel in steam boilers.

## Brucine method

## 1. Summary of method

The reaction between the alkaloid, brucine, and nitrate in acid medium produces a yellow color that may be measured by standard spectrophotometric procedures. Close attention must be given to procedural technique if accuracy and precision are to be obtained. The procedure is similar to that of Jenkins and Medsker (1964).

## 2. Application

This method may be applied to essentially colorless water containing up to 5.0 mg of nitrate per liter. Any significant amount of color should be removed. Samples containing higher concentrations must be diluted.

## 3. Interferences

Organic color, nitrite ion, and all strong oxidizing and reducing agents interfere. The interference by residual chlorine up to 5 mg/l may be eliminated by addition of sodium arsenite, and interference of up to 1 mg/l of nitrite eliminated by use of sulfanilic acid. The interference by chloride is effectively masked by the addition of a large amount of chloride ion to the reaction mixture.

#### 4. Apparatus

4.1 *Water bath*, boiling.

4.2 *Spectrophotometer*, Beckman Model B, or equivalent.

With this instrument, the following operating conditions have been used:

Wavelength.....	410 nm.
Cells.....	23 mm.
Phototube.....	Blue-sensitive.
Initial sensitivity setting.....	2.
Slit width (approximate).....	0.10 mm.

With these operating conditions, the following absorbances have been observed:

NO <sub>3</sub> (mg)	Absorbance
0.005.....	0.115
.010.....	.220
.020.....	.440
.030.....	.640
.040.....	.800
.050.....	.950

#### 5. Reagents

5.1 *Brucine-sulfanilic acid* reagent: Dissolve 1 g brucine sulfate (CAUTION: Very poisonous) and 0.1 g sulfanilic acid in 70 ml hot demineralized water. Add 3 ml concentrated HCl (sp gr 1.19), cool, and dilute to 100 ml. This solution is stable for several months. The pink color that develops does not affect its usefulness.

5.2 *Nitrate* standard solution I, 1.00 ml = 1.00 mg NO<sub>3</sub>: Dissolve 1.631 g KNO<sub>3</sub>, dried overnight over concentrated H<sub>2</sub>SO<sub>4</sub>, in demineralized water and dilute to 1,000 ml.

5.3 *Nitrate* standard solution II, 1.00 ml = 0.010 mg NO<sub>3</sub>: Dilute 10.0 ml nitrate standard solution I to 1,000 ml with demineralized water.

5.4 *Sodium chloride* solution: Dissolve 300 g NaCl in 1 liter demineralized water.

5.5 *Sulfuric acid*, 29*N*: Add 500 ml concentrated H<sub>2</sub>SO<sub>4</sub> (sp gr 1.84) to 125 ml demineralized water.

#### 6. Procedure

6.1 Pipet a volume of sample containing less than 0.05 mg NO<sub>3</sub> (10.0 ml maximum) into a 23-mm absorption cell and dilute to 10.0 ml.

6.2 Prepare a demineralized-water blank and sufficient standards and adjust the volume of each to 10.0 ml.

6.3 Add 2.0 ml sodium chloride solution, and mix well by swirling.

6.4 Place the absorption tubes into a cold-water bath (15°–20°C) and add 10.0 ml 29*N* H<sub>2</sub>SO<sub>4</sub>. Mix well by swirling, return to cold-water bath, and allow the contents of the tubes to cool to water-bath temperature.

6.5 Add 0.5 ml brucine-sulfanilic acid solution and mix thoroughly.

NOTE.—If a deep-pink color forms immediately upon addition of the brucine-sulfanilic acid reagent, it is an indication of a high nitrate concentration—exceeding the range of the method. Such sample aliquots must be discarded at this point and the samples reanalyzed, using a smaller aliquot.

6.6 Remove the rack of tubes from the cold-water bath and place in a boiling-water bath for 20 min. The water bath must be sufficiently large so that boiling does not cease when the tubes are placed in it. This step is critical. All tubes must be heated uniformly.

6.7 Remove the tubes from the boiling-water bath and immerse them in the cold-water bath. Allow to cool before proceeding. This inhibits any further color change. The cold-water bath must be sufficiently large to cool all tubes uniformly. Circulation of water in the bath is desirable.

6.8 Determine the absorbance of the sample and standards against the blank within 1 hr.

#### 7. Calculations

7.1 Determine the mg NO<sub>3</sub> in the sample from a plot of absorbances of standards.

$$7.2 \text{ NO}_3 \text{ in mg/l} = \frac{1,000}{\text{ml sample}} \times \text{mg NO}_3 \text{ in sample.}$$

7.3 To convert NO<sub>3</sub> to N, multiply by 0.2259.

#### 8. Report

Report NO<sub>3</sub> concentrations as follows: Less than 10 mg/l, one decimal; 10 mg/l and above, two significant figures.

#### 9. Precision

Single-laboratory analysis of two test samples resulted in mean values of 0.9 and 2.9 mg/l, and standard deviations of 0.09 and 0.11 mg/l, respectively.

### Reduction method

Samples containing more than 30 mg/l of  $\text{NO}_3^-$  may be analyzed by reduction using Devarda's alloy, distillation of the resulting  $\text{NH}_3$ , and titration with standard  $\text{H}_2\text{SO}_4$  solution. Details of the procedure are given by Blaedel and Meloche (1963) and by Kolthoff and Sandell (1952). For high concentrations of nitrate, the method yields results which are comparable in accuracy to those obtained by the brucine method.

### References

- Blaedel, W. J., and Meloche, V. W., 1963, *Elementary quantitative analysis—Theory and practice* [2d ed.]: New York, Harper & Row, 826 p.
- California State Water Quality Control Board, 1963, *Water quality criteria*: Pub. 3-A, p. 226.
- Comly, H. H., 1945, Cyanosis in infants caused by nitrates in well water: *Am. Med. Assoc. Jour.*, v. 129.
- Jenkins, D., and Medsker, L. L., 1964, Brucine method for determination of nitrate in ocean, estuarine, and fresh waters: *Anal. Chemistry*, v. 36, p. 610.
- Kolthoff, I. M., and Sandell, E. B., 1952, *Textbook of quantitative inorganic analysis* [3d ed.]: New York, Macmillan Co., 759 p.

## Nitrogen, nitrite

Nitrite is unstable in the presence of oxygen and is, therefore, absent or present in only minute quantities in most natural waters under aerobic conditions. The presence of nitrite in water is sometimes an indication of organic pollution.

Recommended tolerances of nitrite in domestic water supplies differ widely. A generally accepted limit is 2 mg/l, but as little as 0.1 mg/l has been proposed (California State Water Quality Control Board, 1963). Nitrite is undesirable in water used in dyeing wool and silk and in brewing.

### Diazotization method

#### 1. Summary of method

Nitrite is diazotized with sulfanilamide, and the resulting diazo compound is coupled with 1-naphthylethylenediamine dihydrochloride to form an intensely colored red dye (Rider and Mellon, 1945). The absorbance of the dye is proportional to the amount of nitrite present (Fishman and others, 1964).

#### 2. Application

This method may be applied to samples containing less than 4 mg nitrite per liter. Samples containing higher concentrations must first be diluted.

#### 3. Interferences

None of the substances commonly occurring in natural water interferes with this method.

#### 4. Apparatus

*Spectrophotometer*, Beckman Model B, or equivalent.

With this instrument, the following operating conditions have been used:

Wavelength.....	535 nm.
Cells.....	10 mm.
Phototube.....	Blue-sensitive.
Initial sensitivity setting.....	2.
Slit width.....	0.08 mm.

With these operating conditions, the following absorbances have been observed.

$\text{NO}_2$ (mg)	Absorbance
0.05.....	0.53
.10.....	1.06
.15.....	1.59
.20.....	2.04

#### 5. Reagents

5.1 *Formic acid*, 87–90 percent.

5.2 *1-naphthylethylenediamine dihydrochloride* solution: Dissolve 0.5 g 1-naphthylethylenediamine dihydrochloride in 100 ml demineralized water. Store in refrigerator.

5.3 *Nitrite standard solution I*, 1.00 ml = 1.00 mg  $\text{NO}_2$ : Dissolve 1.850  $\text{KNO}_2$  in demineralized water and dilute to 1,000 ml.

5.4 *Nitrite standard solution II*, 1.00 ml = 0.010 mg  $\text{NO}_2$ : Dilute 10.0 ml nitrite standard solution I to 1,000 ml with demineralized water.

5.5 *Sulfanilamide* solution: Dissolve 0.5 g sulfanilamide in 100 ml demineralized water.

#### 6. Procedure

6.1 Pipet a volume of sample containing less than 0.20 mg  $\text{NO}_2$  (50.0 ml maximum) into a 100-ml volumetric flask and adjust the volume to 50 ml with demineralized water.

6.2 In a similar manner, prepare a blank and sufficient standards containing 0.00–0.20 mg NO<sub>2</sub>, and adjust the volume of each to 50 ml with demineralized water. Place in ice bath and allow to cool for about 2 min.

6.3 Add successively, while in the ice bath, and mixing thoroughly after each addition: 1.0 ml sulfanilamide solution, 4.0 ml formic acid, and 1.0 ml 1-naphthylethylenediamine dihydrochloride solution.

6.4 Remove the flasks from the ice bath and allow at least 15 min for maximum color development. Adjust each to exact volume with demineralized water. Mix thoroughly and measure the absorbance of samples and standards against the blank.

#### 7. Calculations

7.1 Determine the mg NO<sub>2</sub> in the test samples from a plot of absorbances of standards.

$$7.2 \text{ NO}_2 \text{ in mg/l} = \frac{1,000}{\text{ml aliquot}} \times \text{mg NO}_2 \text{ in}$$

sample.

7.3 To convert NO<sub>2</sub> to N, multiply by 0.3043.

#### 8. Report

Report NO<sub>2</sub> concentrations as follows: Less than 1.0 mg/l, two decimals; 1.0 mg/l and above, two significant figures.

#### 9. Precision

No precision data are available.

#### References

- California State Water Quality Control Board, 1963, Water quality criteria: Pub. 3-A, p. 226.  
 Fishman, M. J., Skougstad, M. W., and Scarbro, G. F., 1964, Diazotization method for nitrate and nitrite: Am. Water Works Assoc. Jour., v. 56, p. 633.  
 Rider, B. F., and Mellon, M. G., 1945, Colorimetric determination of nitrites: Indus. Eng. Chemistry, Anal. Ed., v. 18, p. 76.

### Nitrogen, organic

Organic nitrogen includes all nitrogenous organic compounds, such as amino acid, polypeptides, and proteins. It is present naturally in all surface waters as the result of inflow of nitrogenous products from the watershed and the normal biological life of the stream. Effluents of sewage

and waste from slaughter houses and chemical plants often contain nitrogen in varying combinations. Organic nitrogen in unpolluted ground water is usually very low.

Organic nitrogen is not pathologically significant but is sometimes an indication of pollution. Organic nitrogen is important to considerations involving aquatic biology.

#### Kjeldahl method

##### 1. Summary of method

Organic nitrogen is degraded to the ammonium ion by digestion with sulfuric acid in the presence of copper sulfate, which acts as a catalyst. The solution is made alkaline with sodium hydroxide, and the free ammonia is distilled off and Nesslerized. The color developed is proportional to the organic nitrogen content.

Additional information on the principle of the determination is given by Kolthoff and Sandell (1952, p. 537).

##### 2. Application

This method may be applied to most natural water containing less than 2 mg of nitrogen per liter. Higher concentrations must be reduced by dilution.

##### 3. Interferences

Nitrate and nitrite do not interfere. The effect of ammonium ions and ammonia is strictly additive. Therefore, the organic nitrogen is normally determined on the residue of the ammonia nitrogen determination.

Calcium, magnesium, iron, and sulfide interfere with the Nesslerization, but the interference of the metals is eliminated by the distillation. Sulfides interfere and must be precipitated in the distillation flask with a little lead carbonate before addition of sodium hydroxide.

Some organic compounds may distill over with the ammonia and form colors with Nessler reagent which cannot be satisfactorily read with the spectrophotometer. Under such conditions, the sample should be distilled into H<sub>3</sub>BO<sub>3</sub> and titrated with standard H<sub>2</sub>SO<sub>4</sub>.

##### 4. Apparatus

4.1 *Kjeldahl distillation apparatus*, 1,000-ml flasks.

4.2 *Spectrophotometer*, Beckman Model B, or equivalent.

With this instrument, the following operating conditions have been used:

Wavelength.....	425 nm.
Cells.....	40 mm.
Phototube.....	Blue-sensitive.
Blank.....	Ammonia-free water plus reagents.
Initial sensitivity setting.....	1.
Slit width (approximate).....	0.3 mm.

With these operating conditions, the following absorbances have been observed:

N (mg)	Absorbance
0.02.....	0.24
.04.....	.47
.06.....	.70
.10.....	1.16

#### 5. Reagents

5.1 *Ammonium chloride* standard solution I, 1.00 ml = 1.00 mg N: Dissolve 3.819 g  $\text{NH}_4\text{Cl}$ , dried overnight over sulfuric acid, in ammonia-free water and dilute to 1,000 ml.

5.2 *Ammonium chloride* standard solution II, 1.00 ml = 0.010 mg N: Dilute 10.00 ml  $\text{NH}_4\text{Cl}$  standard solution I to 1,000 ml with ammonia-free water. Prepare fresh.

5.3 *Borate* buffer solution: Dissolve 9.54 g  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  in ammonia-free water. Adjust the pH to 9.5 with 1M NaOH (approx 15 ml) and dilute to 1 liter with ammonia-free water.

5.4 *Boric acid* solution: Dissolve 20 g  $\text{H}_3\text{BO}_3$  in ammonia-free water and dilute to 1 liter.

5.5 *Copper sulfate* solution: Dissolve 10 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in ammonia-free water and dilute to 100 ml.

5.6 *Nessler* reagent (CAUTION:  $\text{HgI}_2$  is a deadly poison, and the reagent must be so marked): Dissolve 100 g  $\text{HgI}_2$  and 70 g KI in a small volume of ammonia-free water. Add this mixture slowly, with stirring, to a cooled solution of 160 g NaOH in 500 ml ammonia-free water and dilute to 1 liter. Allow the reagent to stand at least overnight and filter through a fritted-glass crucible.

5.7 *Sodium hydroxide* solution, 10N: Dissolve 400 g NaOH in ammonia-free water and dilute to 1 liter.

5.8 *Sulfuric acid*, concentrated (sp gr 1.84).

#### 6. Procedure

All glassware should be rinsed with ammonia-free water.

6.1 Free the distillation apparatus of ammonia by boiling ammonia-free water until the distillate shows no trace using Nessler reagent (CAUTION: Deadly poison).

6.2 The residue from the ammonia nitrogen determination may be used for this determination. Alternatively, buffer a volume of sample containing less than 1.0 mg organic nitrogen (500.0 ml maximum) to pH 9.5 with borate buffer solution and evaporate to approximately 20 percent of original volume to drive off ammonia.

6.3 Cool; add 10 ml concentrated  $\text{H}_2\text{SO}_4$  and 1 ml  $\text{CuSO}_4$  solution.

6.4 Digest under a hood until copious fumes are given off and the liquid becomes colorless or pale yellow.

6.5 Cool and dilute to approximately 300 ml with ammonia-free water.

6.6 Add 50 ml 10N NaOH cautiously down the side of the flask.

6.7 Immediately connect the flask to the distillation apparatus, and cautiously mix the contents by swirling gently.

6.8 Distill at a rate of no more than 10 ml nor less than 6 ml per minute; catch the distillate in a 500-ml volumetric flask containing 50 ml boric acid solution. The tip of the delivery tube must be below the surface of the boric acid.

6.9 Collect approximately 250 ml distillate, dilute to 500 ml with ammonia-free water, and mix.

6.9.A *Nesslerization procedure*. Proceed as directed in "Nitrogen, Ammonia," steps 6.6.A.1-6.6.A.5.

6.9.B *Titration procedure*. Proceed as directed in "Nitrogen, Ammonia," step 6.6.B.1.

#### 7. Calculations

##### 7.A *Nesslerization procedure*

7.A.1 Determine a reagent blank for each new batch of  $\text{H}_2\text{SO}_4$  by taking 300 ml ammonia-free water through the entire procedure:

mg reagent blank = mg N per 10 ml  $\text{H}_2\text{SO}_4$

$$\times \frac{\text{ml aliquot}}{\text{ml distillate}}$$

7.A.2 Determine the mg N in the aliquot from a plot of absorbances of standards.

#### 7.A.3

Organic nitrogen as N, in mg/l

$$= \frac{1,000}{\text{ml sample}} \times \frac{500}{\text{ml aliquot}} \times [(\text{mg N in aliquot}) - (\text{mg reagent blank})].$$

#### 7.B Titration procedure

7.B.1 Ammonia nitrogen as N, in mg/l

$$= \frac{V_a \times N_a \times 14,000}{V_s},$$

where

$V_a$  = ml standard  $\text{H}_2\text{SO}_4$  used in titration of sample minus ml used to titrate blank,

$N_a$  = normality of standard  $\text{H}_2\text{SO}_4$ , and

$V_s$  = ml of original sample used for distillation.

7.C Ammonia nitrogen as  $\text{NH}_4^+$ , in mg/l = mg/l as N  $\times 1.288$ .

Ammonia nitrogen as free  $\text{NH}_3$ , in mg/l = mg/l as N  $\times 1.216$ .

#### 8. Report

Report organic nitrogen concentrations as follows: Less than 1.0 mg/l, two decimals; 1 mg/l and above, two significant figures.

#### 9. Precision

No precision data are available.

#### Reference

Kolthoff, I. M., and Sandell, E. B., 1952, *Textbook of quantitative inorganic analysis* [3d ed.]: New York, Macmillan Co., 759 p.

## Oxygen demand, chemical (COD)

The oxygen-demand determination is a measure of the readily oxidizable material in the water, and it furnishes an approximation of the minimum amount of organic and reducing material present. In reality, the term "chemical oxygen demand" is defined by the method used for its determination. In the method given below it is defined as the amount of oxygen used by the sample when refluxed 2 hr with an excess of acid-potassium dichromate solution. The determined value may correlate with natural-water color or with ear-

bonaceous organic pollution from sewage or industrial wastes.

Normal, unpolluted river waters generally have a COD from about 10 to 30 mg/l; mildly polluted river waters, 25 to 50 mg/l; and domestic sewage about 250 mg/l (R. C. Kroner, written commun., 1970).

Tolerances for oxygen-demand values in feed water for low- and high-pressure boilers are 15 and 3 mg/l, respectively. Wash water containing more than 8 mg/l has been reported to impart a bad odor to textiles; concentrations for water used in beverages and brewing range from 0.5 to 5.0 mg/l (California State Water Quality Control Board, 1963).

### Dichromate oxidation method

#### 1. Summary of method

Organic and other oxidizable material is oxidized by refluxing with standard acid-dichromate solution in the presence of silver sulfate catalyst. The excess dichromate is titrated with standard ferrous ammonium sulfate, using orthophenanthroline ferrous complex as indicator (American Society for Testing and Materials, 1968).

#### 2. Application

This method can be used for analysis of natural waters and industrial wastes containing less than 2,000 mg/l chloride ion and more than 50 mg/l chemical oxygen demand (COD). Samples containing less than this amount should be analyzed as directed in step 6.9. COD values for waters containing more than 2,000 mg/l of chloride ion should be corrected as indicated in step 6.10.

#### 3. Interferences

Reducing substances such as ferrous iron and chlorides interfere since they are oxidized. Chlorides constitute by far the largest and most common interference, being quantitatively oxidized by dichromate in acid solution. One mg/l  $\text{Cl}^-$  is equivalent to 0.226 mg/l COD. To eliminate chloride interference, mercuric sulfate is added to the sample to form a soluble mercuric chloride complex.

Care should be taken to prevent heating of the sample during addition of reagents to minimize loss of volatile constituents.